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Direct Shoot Regeneration and Plantlets Development from Nodal Explant of *Canthiumcoromandelicum* (Burm. f.) Alston

Priyadarshini S. Ekambe¹, Babasaheb S. Surwase²,

Ph.D. Student, School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded,
Maharashtra, India- 431606¹

Associate Professor, School of Life Sciences, Swami Ramanand Teerth Marathwada University,
Nanded, Maharashtra, India- 431606²

ABSTRACT: A protocol for direct shoot regeneration and plantlets development from nodal explant of *Canthiumcoromandelicum* (Burm. f.) Alston a medicinal plant belonging to family Rubiaceae was developed. High frequency *in vitro* direct multiplication protocol was standardized from the nodal explants derived from *in vitro* raised 30 days old seedlings. Maximum shoot multiplication frequency was obtained on Murashige&Skoog medium supplemented with 3 mg/l Kinetin (Kn) alone. The elongated shoots were subcultured on ¼ MS with 3 mg/l NAA for rooting. The *in vitro* raised plantlets were acclimatized in green house and progressively transplanted to natural conditions with 70% survival.

KEYWORDS: Kn; *Canthiumcoromandelicum* (Burm. f.) Alston; Micropropagation; Murashige&Skoog medium; NAA.

I. INTRODUCTION

Canthiumcoromandelicum (Burm. f.) Alston is medicinally important plant belonging to family Rubiaceae and known by various synonyms like *Canthium parviflorum*, *Plectronia parviflora*, *Paederia valli-kara*, *Webera tetrandra*. It is native of India found mainly in coromandelicum region. It is the shrub with spreading branches, a thorny shrub found throughout Indian forest and dry plains. Its leaves are simple, small, obviate, opposite with interpetiolar stipules linear and axillary spines. It possesses wound healing, diuretic, antioxidant properties. Also shows oral hypoglycaemic and hypocholesterolaemic activity [1, 2]. Due to unrestricted large scale exploitation of this natural resource, the wild stock of this species has been markedly depleted. Plant tissue culture promises the continuous supply of raw material required for herbal industry without seasonal constraints. In the present communication, we report a protocol for regeneration of plants from nodal explants. This is the first report of direct regeneration of *C. coromandelicum* plantlets through tissue culture. This protocol may be useful in conservation of this plant species.

II. MATERIALS AND METHODS

Plant Material

Collection of fruits of *Canthiumcoromandelicum* was done from 'Udgir' in Latur district, India. It was authenticated by Dr. R. M. Mulani, School of Life Sciences, S.R.T.M. University, Nanded (MS), India.

Preparation of Explant

The shade dried seeds were used for seed germination. Seeds were washed with running tap water to remove adhere dust, dirt particles. Dip it in 1% Sodium hypochlorite solution for 1-2 min and washed with sterile distilled water. After that they were treated with 70% v/v ethanol for 1 min. Again washed with sterile distilled water. Seeds were treated with 1% HgCl₂ for surface sterilization for 1 min. Finally they were washed with sterile distilled water for thrice to

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remove all chemicals. To break the seed dormancy, seed coat was mechanically damaged. Seeds were inoculated on Knudson's C medium for seed germination. After four weeks, there was development of seedlings observed. These were used as a source of explants. Nodal explants were prepared by cutting the parts in aseptic conditions.

Culture Media & Conditions

For direct regeneration of shoots nodal explant inoculated in Murashige&Skoog (MS, 1962) medium supplemented with 0.9% (w/v) Agar-agar, 3% sucrose with cytokinin like Kinetin (1-10 mg/l). The pH of the medium was adjusted to 5.8. Molten medium (15 ml) was poured into each test tube and was autoclaved at 15 lbs and 121°C for 15-20 mins. After solidifying of medium explant was inoculated and incubated at 25±2°C at a relative humidity of 70-80% under 16 h photoperiod of 2000 lux light intensity provided by cool white fluorescent tubes. For each treatment 20 replicates were used. The growing explants were sub cultured after every 2 weeks. All the experiments were repeated in triplicate.

Rooting and Acclimatization

For induction of roots, the 3 cm elongated shoots were transferred to ¼ MS medium with different concentrations of Naphthalene 3- acetic acid (NAA). The rooted plantlets were transferred to plastic pots containing a mixture of sterile soil and sand (1:1) in the lab conditions. Humidity was maintained at 70- 80 % by covering the plants with polythene bags. The polythene bags were progressively removed to reduce the humidity. After acclimatization for 15 days, plantlets were transferred to green house for one month with an average temperature of 25 ±2°C. Following hardening, the plantlets were transplanted to pots containing garden soil alone.

III. EXPERIMENTAL RESULTS

Direct regeneration of plantlets was observed in many plants. Different parts of plants used as explant for direct regeneration. Nodal explants were inoculated on MS medium containing different concentrations of Kinetin for optimization of cytokinin concentration and to elicit the best regeneration response. Induction of multiple shoots took place in all concentrations of Kinetin used. Maximum shoot multiplication i.e. 7.16±0.47 shoots per explants was achieved on MS medium containing 3 mg/l Kinetin with 70% response. The effect of different media like Full MS, Half MS medium alone and in combination with Kinetin on *in vitro* direct multiplication of shoots were shown in Table 1. Either decrease or increase in concentration of Kinetin reduced the shoot number, percentage of response and an average shoot length.

Table 1.Effect of Full MS, Half MS medium in combination with Kinetin on *in vitro* direct multiplication of shoots by using nodal explant of *C. coromandelicum*.

Medium	Growth Hormones Kinetin (mg/l)	Percentage Response (%) / explant	Shoot Number/ Explant Mean ± SE
Half MS	-	30	3.33±0.71
Full MS	-	30	3.33±0.71
Full MS	1	40	3.16±0.30
Full MS	2	50	4.83±0.47
Full MS	3	70	7.16±0.47
Full MS	4	60	6.00±0.73
Full MS	5	50	5.50±0.88
Full MS	6	50	5.33±0.55
Full MS	7	50	4.16±0.40
Full MS	8	50	4.83±0.47
Full MS	9	40	4.50±0.42
Full MS	10	40	4.16±0.40

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There was some reported work on direct shoot regeneration of medicinal plants. By eliminating unnecessary callus formation in *Phalaenopsis* orchids direct shoot regeneration from nodes was also reported [3]. *In vitro* shoot induction and plant regeneration from nodal segments of *Balanitesaegyptiaca* on Murashige and Skoog (MS) medium fortified with 6-benzyladenine (BA), thidiazuron (TDZ) and kinetin (Kin) was also observed [4]. A rapid and efficient protocol for high frequency direct shoot proliferation was observed in *Andrographispaniculata*, a valuable medicinal plant by using nodal explant[5]. Direct shoot regeneration from nodal explant also reported in *Sidacordifolia* Linn. [6], *Pogostemoncablin* Benth. [7]. Germination and development of 30 days old seedling, initiation of nodal explant to elongation of 2-3 cm long shoots were depicted in Fig. 1.

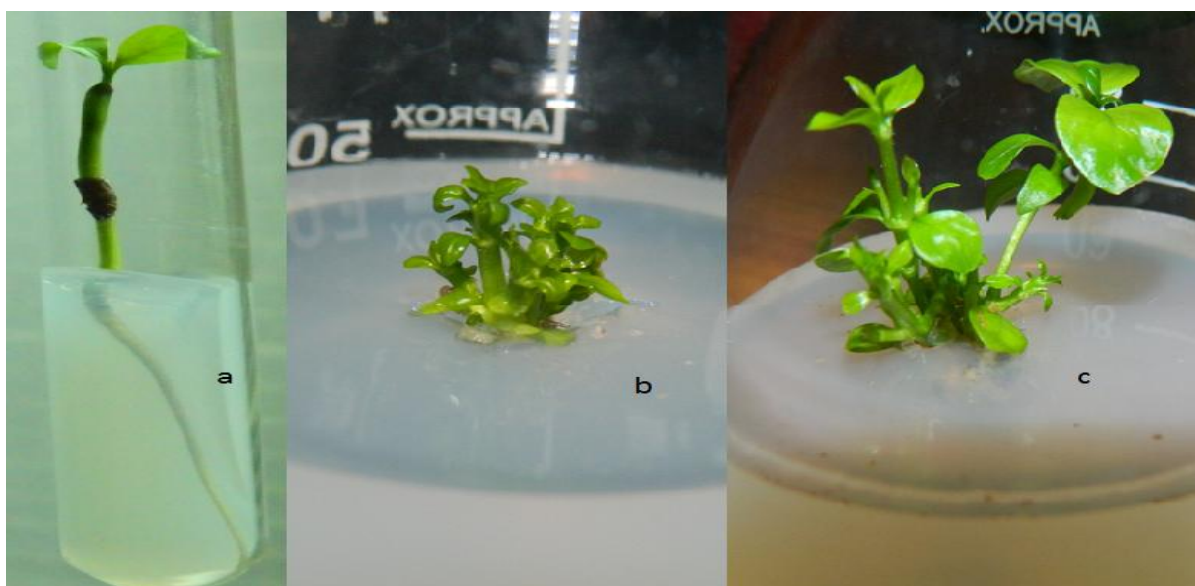


Fig. 1. (a) Seed germination and seedling development of *C. coromandelicum* on Knudson’s medium. (b) Initiation of shoot (c) Induction of multiple shoots.

For rooting purpose two week old healthy shoots with 3-4 cm in height were cut and inoculated on ¼ MS medium containing different concentrations of NAA for induction of roots. Table 2 showed *in vitro* root regenerated shoots of *C. coromandelicum* on full, half and ¼ strength MS medium alone and supplemented with different concentrations of NAA. Amongst different concentrations of NAA tried 3 mg/l concentration was the best and showed maximum root number i.e. 7.16 ± 0.16 per shoot.

Table 2. *In vitro* root regenerated shoots of *C. coromandelicum* on full, half and ¼ strength MS medium supplemented with different concentrations of NAA.

Medim	Growth Hormone (mg/l) NAA	Percentage Response (%)	Average No. of Roots/ Explant (Mean± SEM)
MS	-	30	1.08±0.08
½ MS	-	30	2.00±0.25
¼ MS	-	50	2.00±0.25
¼ MS	1	60	4.83±0.47
¼ MS	2	70	5.00±0.25
¼ MS	3	90	7.16±0.16
¼ MS	4	80	5.33±0.21
¼ MS	5	80	5.16±0.30

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NAA is widely used for induction of roots on regenerated shoots of medicinal plants like *Adathodavasica*[8], *Hemarthriacompressa*[9], *Tavernieracuneifolia*[10]. Similarly, IBA promoting rooting in *Hebe buchananii* (Hook) [11]. Direct multiple shoots were regenerated successfully and a single shoot was transferred on rooting media. 2- 3 cm long plantlet with 4- 5 leaves and roots then acclimatized successfully (Fig. 2).



Fig. 2. (d) Development of shoots after four weeks. (e) *In vitro* regenerated roots. (f) Acclimatization of plantlets. The plantlet is successfully transferred to soil after hardening with a 70% survival rate. Acclimatization is the final step in a successful micropropagation system. During this stage plants have to adapt to the new environment of greenhouse or field. The plantlets usually need some weeks of acclimation in shade with the gradual lowering of air humidity [12].

IV. CONCLUSION

In our study it was observed that the combination of MS medium with Kinetin generated direct multiple shoots from nodal explant obtained from seedlings of *Canthiumcoromandelicum*. And these regenerated shoots were showed rooting on ¼ MS medium supplemented with NAA. Acclimatization rate was 70% observed.

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BIOGRAPHY

My name is Priyadarshini S. Ekambe. I was completed my M.Sc. (Biotech.) from Swami RamanandTeerthMarathwada University, Nanded- 431606, Maharashtra, India. And now I am working as a Ph.D. (Biotechnology) Student under the guidance of Dr. B. S. Surwase (Associate Professor) at School of life sciences, Swami RamanandTeerthMarathwada University, Nanded-431606, Maharashtra, India.